Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2931	chitinase$1 or chitotriosidase$1	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:04
L2	47	1 near4 human	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:11
L3	75	1 same (culture adj medi$4)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:20
L4	101	1 same (cosemetic$1 or dental or toothpaste$1 or food)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:21
L5	230	1 same antifung$	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:21
L6	19	5 same (human or mammal$)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:22
L7	61185	drug same (deliver$ or release or implant)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:23
L8	13	1 same 7	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:23

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2931	chitinase$1 or chitotriosidase$1	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:04
L2	47	1 near4 human	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:11

PGPUB-DOCUMENT-NUMBER: 20040253224

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040253224 A1

TITLE: Mammalian mucinase, its recombinant production, and its use in therapy or prophylaxis against diseases in which mucus is involved or infectious diseases

PUBLICATION-DATE: December 16, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Gerardus Aerts, Johannes	Abcoude		NL	
Maria Franciscus	Amsterdam		NL	
Boot, Rolf Gabriel				

APPL-NO: 10/ 787845

DATE FILED: February 26, 2004

RELATED-US-APPL-DATA:

child 10787845 A1 20040226

parent continuation-of 10004219 20011102 US ABANDONED

US-CL-CURRENT: 424/94.61, 435/200

ABSTRACT:

The invention provides a mammalian mucinase capable of hydrolyzing mucin. The mucinase is, among other things, suitable for counteracting diseases in which mucus is involved, such as cystic fibrosis, COPD, asthma, bronchitis, tuberculosis, tumors with altered mucus expression, and mucus-containing pathogens. The invention also provides a pharmaceutical composition comprising an effective amount of the mucinase and a method of therapeutic or prophylactic treatment of an individual against such a disease. Methods for obtaining the mucinase are also herewith provided, as well as nucleic acids encoding all or part of the mucinase. In one aspect, the invention provides a diagnostic kit comprising a mucinase, a mucinase-specific antibody, a mucinase-derived peptide, and/or nucleic acid encoding all or part of the mucinase.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application is a continuation of application Ser. No. 10/004,219, filed Nov. 2, 2001, pending, the contents of the entirety of which are incorporated herein by this reference.

---------- KWIC ---------

Brief Description of Drawings Paragraph - DRTX (3):

[0048] FIG. 2. Degradation products with colloidal chitin as substrate. The FACE technique (described in experimental procedures) was used to visualize the cleavage products of recombinant human chitotriosidase and recombinant

mouse AMCase using colloidal chitin as substrate. Lane 1, no enzyme added. Lane 2, products formed after incubation with 50 kDa recombinant human chitotriosidase with chitin. Lane 3, products formed with recombinant mouse AMCase and chitin. Lane 4, human chitotriosidase incubated without substrate. Lane 5, mouse AMCase incubated without substrate. Marker lane is indicated with M (sugar polymers are indicated on the right-hand side).

Brief Description of Drawings Paragraph - DRTX (5):

[0050] Panel A: Purified recombinant human chitotriosidase and mouse AMCase were separated on a 12.5% SDS-PAGE gel in the presence or absence of a reducing agent and visualized by silver staining as described in experimental procedures (panel A). Lane 1, recombinant mouse AMCase under reducing conditions. Lane 2, recombinant human chitotriosidase under reducing conditions. Lane 3, recombinant human chitotriosidase under non-reducing conditions. Lane 4, recombinant mouse AMCase under non-reducing conditions. M indicates the molecular weight standards (mass (kDa) indicated at the left-hand side).

Brief Description of Drawings Paragraph - DRTX (6):

[0051] Panel B: The same purified recombinant enzymes as described in panel A were separated on a 10% SDS-PAGE gel containing glycol-chitin as described in experimental procedures. Chitinolytic activity was visualized as clearing zones in the gel. Lane 1, recombinant human 39 kDa chitotriosidase. Lane 2, recombinant human 50 kDa chitotriosidase. Lane 3, recombinant mouse AMCase (mass (kDa) indicated at the right-hand side).

Brief Description of Drawings Paragraph - DRTX (8):

[0053] Panel A: pH activity profile of the different chitinases. The pH optima were determined by monitoring enzyme activity at the indicated pH in McIlvaine buffer. Purified human recombinant chitotriosidase (closed lozenge), purified mouse AMCase (closed circle).

Brief Description of Drawings Paragraph - DRTX (9):

[0054] Panel B: Effects of acidic pre-incubation. Purified recombinant human chitotriosidase and mouse AMCase were pre-incubated for 30 minutes at the indicated pH in McIlvaine buffer prior to enzyme activity measurement at the assay pH (see experimental procedures). Activity prior to incubation at the indicated pH is defined as 100%.

Brief Description of Drawings Paragraph - DRTX (10):

[0055] Panel C: Precipitation by trichloroacetic acid (TCA). Purified recombinant human chitotriosidase and mouse AMCase were incubated with the indicated percentages of TCA. The amount of remaining enzyme activity after centrifugation is shown as a percentage of initial amounts.

Brief Description of Drawings Paragraph - DRTX (16):

[0061] FIG. 8. Amino acid sequence comparison of mature (without signal peptide) human (h) (SEQ ID NO:14) and mouse (m) (SEQ ID NO:9) AMCase and human chitotriosidase (SEQ ID NO:10). Residues conserved among at least two out of the three sequences are in bold.

Detail Description Paragraph - DETX (51):

[0111] Our findings demonstrate that AMCase in mammalians is distinct from chitotriosidase: the newly discovered, discrete enzyme is referred to as acidic

mammalian chitinase or AMCase. AMCase is also present in man. Screening the human EST database at the NCBI with the acidic mouse chitinase cDNA revealed the presence of a human EST clone (oq35c04.s1, Genbank acc. no. AA976830) that is highly homologous to the acidic mouse chitinase. The tissue distribution of the human mRNA was examined using a human Masterblot (Clontech). The expression pattern of this mRNA is similar to the expression pattern of the acidic mouse chitinase (FIG. 5), being highly expressed in the stomach and at a lower level in the lung. Using degenerate oligonucleotides directed against members of the chitinase family, we were able to amplify other regions of the human acidic chitinase, generating enough information to clone the full-length human acidic chitinase cDNA. Screening the Genbank database using the full-length human cDNA revealed that it was almost identical to TSA1902-L and TSA1902-S from a lung cDNA library described by Saito et al. (Saito et al., 1999). These two sequences are most probably splice variants of the acidic human chitinase mRNA. Only expression of full-length human AMCase cDNA in COS cells led to the production of a protein with chitinolytic activity. Sequence comparison of the human acidic chitinase and the mouse acidic chitinase revealed an 82% identity and a similarity of 86%. (compare Table II (SEQ ID NO:2) and Table III (SEQ ID NO:3)). The catalytic domain of human AMCase is also herewith provided.

Detail Description Paragraph - DETX (62):

[0119] To obtain more insight into the potential occurrence of multiple mammalian chitinases, tissues of mouse and rat were examined for chitinolytic activity using the chitin-like 4-methylumbelliferyl-.beta.-c-hito-oligosaccharide substrates. In extracts of stomach and intestine, a high level of activity was detected, while extracts of lung, tongue, kidney and plasma showed significant but lower activities. Isoelectric focusing (by flatbed isoelectric focusing in granulated Ultrodex gels (Pharmacia) as described by Renkema et al., 1995) of a mouse lung extract revealed a major peak of chitinolytic activity with pI 4.5 while minor peaks were found with pIs 5.5-6.5 (FIG. 1). Extracts of other mouse and rat tissues showed similar profiles of chitinolytic activity upon isoelectric focusing. The observed rodent chitinase with acidic isoelectric point (pI 4.5 form) differs strikingly from human chitotriosidase, which has an apparent neutral/basic pI.

Detail Description Paragraph - DETX (64):

[0121] The procedure resulted in a 30,000-fold purification of an apparently homogeneous 50 kDa protein. The specific activity of the purified enzyme was 3.9 nmol 4-methylumbelliferyl-chitotrioside hydrolyzed per mg per hour at pH 5.2, which is almost identical to that of human chitotriosidase.

Detail Description Paragraph - DETX (66):

[0123] Reverse transcription-polymerase chain reaction (RT-PCR) fragments were generated from mouse lung total RNA using degenerate oligonucleotides, as described (Boot et al., 1995). Obtained fragments were cloned in pGEM-T (Promega, Madison, Wis., USA), sequenced and compared with the amino acid sequence established by N-terminal protein sequencing. A comparison with the GenBank mouse EST (expressed sequence tag) database using the Basic local alignment search tool (BLAST) at NCBI (National Center for Biotechnology Information) showed that several EST clones matched the mouse chitinase cDNA sequence, for example, ms33h09.y1 (GenBank Accession Number AI892792). This clone was obtained and sequenced. Anti-sense primers were generated complementary to the most 3' region of the EST clone (A-tail primer: 5'-TTTTGGCTACCAATTTTATTGC-3') (SEQ ID NO:5) and two internal anti-sense primers (MASI : 5'-CAGCTACAGCAGCAGTAACCATC-3') (SEQ ID NO:6) and (MAS2 : 5'-TTCAGGGATCTCATAGCCAGC-3') (SEQ ID NO:7). The MAS1 and MAS2 primers were used to clone the most 5' end of the mouse acidic chitinase cDNA using 5' rapid amplification of cDNA ends (5' RACE) and the Marathon-Ready mouse Lung cDNA kit

(Clontech) according to the instructions of the manufacturer. To obtain the complete coding sequence, a 5' sense primer was generated (MS1: 5'-CGATGGCCAAGCTACTTCTCGT-3') (SEQ ID NO:8). The total cDNA sequence was subsequently generated using MS1 and the A-tail primer. The fragments of two independent PCR's were cloned into pGEM-T (Promega) and the nucleotide sequence of two independent clones from each PCR were sequenced from both strands by the procedure of Sanger using fluorescent nucleotides on an Applied Biosystems (ABI) 377A automated DNA sequencer following ABI protocols. The mouse AMCase protein shows considerable sequence homology to human chitotriosidase. Comparison of the amino acid sequence of both mature proteins revealed an identity of 52% and a similarity of 60%. See, Tables V and VI, below.

Detail Description Paragraph - DETX (68):

[0125] Like the human chitotriosidase, the mouse enzyme is predicted to contain an N-terminal catalytic domain of about 39 kDa, a hinge region and a C-terminal chitin binding domain (see, Table IV, above). The mouse AMCase, like chitotriosidase, is predicted to lack N-linked oligosaccharides, explaining the observed absence of binding to Concanavalin A (data not shown). The apparent molecular masses of identically produced recombinant human chitotriosidase and recombinant mouse AMCase are comparable when run on an SDS-PAGE gel under reducing conditions. However, under non-reducing conditions, the mouse AMCase migrates significantly slower than the human chitotriosidase (FIG. 3A). Upon gel electrophoresis (under non-reducing conditions) in polyacrylamide gels containing glycolchitin, followed by regeneration of active enzyme and detection of the local digestion of glycolchitin using Calcofluor staining, the mouse AMCase migrates slightly faster than human chitotriosidase (FIG. 3B).

Detail Description Paragraph - DETX (71):

[0128] Screening the GenBank database using the full-length human cDNA revealed that it was almost identical to TSA1902-L (GenBank Accession Number AB025008) and TSA1902-S (GenBank Accession Number AB025009) from a lung cDNA library described by Saito et al. (Saito et al., 1999). These two sequences are most probably splice variants of the human acidic chitinase mRNA. Only expression of full-length human AMCase cDNA in COS-1 cells led to the production of a protein with chitinolytic activity (data not shown). Sequence comparison of the human acidic chitinase (see, Table VII, below) and the mouse acidic chitinase (see, Table V, above) revealed an 82% identity and a similarity of 86%.

Detail Description Paragraph - DETX (75):

[0130] Another major difference between human chitotriosidase and the mouse AMCase is revealed by comparison of RNA expression patterns. Total RNA was isolated using RNAzol B (Biosolve, Barneveld, The Netherlands) according to the instructions of the manufacturer. Northern blots, using 15 .mu.g of total RNA, were performed as described (Boot et al., 1995). Mouse RNA Master Blots (Clontech, Palo Alto, Calif., USA) were used to examine the tissue distribution of transcripts according to the instructions of the manufacturer. The following probes were used: the full-length mouse acidic chitinase cDNA and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as control. Radiolabeling and hybridization was conducted as described previously (Boot et al., 1995). Quantification of radioactivity was performed using a phosphor imager (Storm phosphor imager, Molecular Dynamics, Sunnyvale, Calif., USA).

Detail Description Paragraph - DETX (76):

[0131] Whereas human chitotriosidase mRNA is mainly found in lymph node, bone marrow and lung, the mouse AMCase mRNA is predominantly found in, of the screened tissues, stomach, submaxillary gland and, at a lower level, in the lung (FIG. 5). Surprisingly, no mouse acidic chitinase mRNA could be detected

in the small intestine. This can be explained by an absence of mRNA, or by mRNA levels in the sample that were too low for detection with the technique used. These results suggest that the protein in the intestine is probably derived from the upper parts of the gastrointestinal tract, such as the stomach.

Detail Description Paragraph - DETX (77):
[0132] In rat tissues, a comparable acidic chitinase was observed. Our findings indicate that the acidic chitinase in rodents is distinct from human chitotriosidase. The discrete enzyme is therefore referred to as acidic mammalian chitinase or AMCase.

Detail Description Paragraph - DETX (82):
[0135] Several different assays revealed that the mouse acidic chitinase is able to degrade chitin and, therefore, has to be considered to be a true chitinase. Crab shell chitin (Poly-[1-4]-.beta.-D-N-acetylglucosamin- e, Sigma) was used as a natural substrate to determine chitinase activity as described (Renkema et al., 1997). The chitin fragments were analyzed by fluorophore-assisted carbohydrate electrophoresis (FACE) as described by Jackson (Jackson 1990). FACE analysis revealed that recombinant mouse chitinase, like chitotriosidase, releases mainly chitobioside fragments from chitin (FIG. 2). Chitinase enzyme activity was determined in another assay with the fluorogenic substrates 4MU-chitobiose (4-methylumbelliferyl .beta.-D-N,N'-diacetylchitobiose, Sigma, St Louis, USA) and 4MU-chitotriose (4-methylumbelliferyl .beta.-D-N,N',N"-triacetyl- chitotriose, Sigma). Assay mixtures contained 0.027 mM substrate and 1 mg/ml of bovine serum albumin (BSA) in McIlvaine buffer (100 mM citric acid, 200 mM sodium phosphate) at the indicated pH. The standard enzyme activity assay for human chitotriosidase with 4MU-chitotriose substrate was performed at pH 5.2, as previously described (Hollak et al., 1994). The standard AMCase enzyme activity assays with 4MU-chitobiose substrate were performed at pH 4.5. Like chitotriosidase and some other non-mammalian chitinases, the mouse acidic chitinase activity in this assay is strongly inhibited (IC.sub.50 of 0.4 .mu.M) by the competitive chitinase inhibitor allosamidin (Milewski et al., 1992, Dickinson et al., 1989, McNab and Glover 1991). Measurements of chitin formation during regeneration of fingal spheroplasts were performed as described by Hector and Braun (Hector and Braun 1986). Briefly, spheroplasts were prepared from the Candida albicans strain CAi-4 (ura3), grown overnight in YPD medium (1% yeast extract, 2% peptone, 2% glucose) at 28.degree. C. Cells were concentrated by centrifugation and incubated with 2.5 mg/ml zymolyase (1 OOT, ICN Immuno Biologicals, Costa Mesa, Calif., USA) in buffer containing 50 mM sodium phosphate pH 7.5, 1.2 M sorbitol and 27 mM .beta.-mercaptoethanol for 60 minutes at 37.degree. C. After extensive washing, spheroplasts were allowed to regenerate in 96-well microtiter plates in regeneration buffer (0.25% (w/v) MES buffer pH 6.7, containing 0.17% (w/v) Yeast Nitrogen Base (without amino acids and ammonium sulfate, Sigma), 0.15% (w/v) ammonium sulfate, 2% (w/v) glucose, 1.2 M sorbitol, 20 .mu.g/ml uridine) at 37.degree. C. Chitinase enzyme preparations were added in 3 .mu.g/ml. After a 2 hour incubation, 50 .mu.l of 300 .mu.g/ml Calcofluor white (Sigma) in 10 mM sodium phosphate buffer pH 7.5 containing 1.2 M sorbitol was added. After 5 minutes, the plates were washed with buffer only and fluorescence was determined using an LS 50 Perkin Elmer fluorometer (excitation 405 nm, emission 450 nm).

Detail Description Paragraph - DETX (121):
[0155] Boot, R. G., Renkema, G. H., Strijland, A., van Zonneveld, A. J. and Aerts, J. M. F. G.: Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. (1995) J. Biol. Chem. 270, 26252-26256.

Detail Description Paragraph - DETX (122):

[0156] Boot, R. G., Renkema, G. H., Strijland, A., van Zonneveld, A. J. and Aerts, J. M. F. G.: Cloning of a cDNA encoding chitotriosidase, a human chitinase produced by macrophages. (1995) J. Biol. Chem. 270, 26252-26256.

Detail Description Paragraph - DETX (132):
[0166] Escott, G. M. and Adams, D. J.: Chitinase activity in human serum and leukocytes. (1995) Infect. Immun. 63(12), 4770-4773.

Detail Description Paragraph - DETX (160):
[0194] Renkema, G. H., Boot, R. G., Muijsers, A. O., Donker-Koopman, W. E. and Aerts, J. M. F. G.: Purification and characterization of human chitotriosidase, a novel member of the chitinase family proteins. (1995) J. Biol. Chem. 270, 2198-2202.

Detail Description Paragraph - DETX (161):
[0195] Renkema, G. H., Boot, R. G., Strijland, A., Donker-koopman, W. E., van den Berg, M., Muijsers, A. O. and Aerts, J. M. F. G.: Synthesis, sorting, and processing into distinct isoforms of human macrophage chitotriosidase. (1997) Eur. J. Biochem. 244, 279-28511.

PGPUB-DOCUMENT-NUMBER: 20040192645

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040192645 A1

TITLE: Amyloid plaque as a target for therapeutics that function by blocking or disrupting chitin synthesis or activity

PUBLICATION-DATE: September 30, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Zipser, Birgit	East Lansing	MI	US	
Huang, Linjuan	East Lansing	MI	US	

APPL-NO: 10/ 795652

DATE FILED: March 8, 2004

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60453432 20030310 US

US-CL-CURRENT: 514/55

ABSTRACT:

Chitin has been discovered to accumulate in the diseased tissue of mammals, including humans, afflicted with a disease characterized by formation of congo red-staining plaques. Such diseases include Alzheimer's disease, spongiform encepalopathies, type II diabetes, atrial amyloidosis, and the like. A method for detecting the chitin in the mammal is described which is useful for diagnosing disease caused by accumulation of the chitin or amyloid plaques comprising chitin in tissue. Further described is a method for treating a disease in the mammal caused by the accumulation of chitin or amyloid plaques comprising chitin by administering a composition which inhibits formation of the chitin or degrades the chitin.

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. provisional application Ser. No. 60/453,432, filed Mar. 10, 2003.

[0002] Reference to a "Computer Listing Appendix submitted on a Compact Disc"

[0003] Not Applicable.

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Summary of Invention Paragraph - BSTX (9):

[0012] While humans do not appear to have a chitin synthase, humans do produce a chitinase. The human chitinase has been described in U.S. Pat. Nos. 6,200,951, 6,399,571, and 6,372,212 to Gray et al., wherein the human chitinase, DNA encoding the chitinase, and fragments of the chitinase for

detecting chitin, binding chitin, and treating fungal infections. These patents provide a detailed background regarding chitinase which enables the present invention.

Summary of Invention Paragraph - BSTX (28):

[0028] The present invention further provides a method for reducing chitin or conjugate thereof in a mammal which comprises administering an effective amount of a chitinase to the mammal. Preferably, the chitinase is human chitinase and the mammal is human.

Detail Description Paragraph - DETX (17):

[0057] Chitinases have been discovered in mammals, including humans, and have been described in U.S. Pat. No. 6,399,571 to Gray et al. and U.S. Pat. Nos. 6,057,142 and 6,301,118 to Aerts. Human chitinase with chitotriosidase activity is expressed by phagocytes (macrophages). A similar chitinase has been found in the lung and an acidic chitinase has been found in the intestine. Chitinases are thought to provide a defense against opportunistic infections by fungi and bacteria. Chitinases may also be involved in removing chitin which may be formed by fluctuations in the ratios of UDP-N-acetylglucosamine and UDP-glucuronic acid in the synthesis of hyaluronic acid. Chitin which is not degraded might accumulate in the body and provide a scaffold or core for assembly of amyloidogenic proteins such as .beta.-protein of Alzheimer's disease into amyloid plaques. Therefore, mutations which cause a decrease or cessation of chitinase activity may be involved in the formation of amyloid plaques because the mutations allow for an accumulation of chitin either from fungal or bacterial infections, from defects in the synthesis of hyaluronic acid which shifts synthesis from hyaluronic acid towards chitin, defects in an exogenouse (bacterial or fungal) or endogenous (not yet discovered in mammals or humans) pathway for the synthesis of chitin which result in an excess accumulation of chitin, or fluctuations in the ratio of UDP-N-acetylglucosamine and UDP-glucuronic acid in the pathway for synthesizing hyaluronic acid which shifts synthesis towards chitin. The chitin then serves as a scaffold for the assembly of amyloid plaques, the assembly of which occurs because of yet unknown defects which cause the particular amyloidogenic proteins comprising the amyloid plaques to self-assemble on the chitin scaffold into the amyloid plaques.

Detail Description Paragraph - DETX (25):

[0065] The present invention can also use labeled chitin binding fragments or degrading proteins as probes to detect chitin directly. Probes specific for chitin include chitin binding lectins such as chitovibrin which is disclosed in U.S. Pat. Nos. 5,914,239 and 6,121,420, both to Laine; chitin binding fragments derived from human chitinase as disclosed in U.S. Pat. Nos. 6,399,571, 6,200,951 and 6,372,212, all to Gray et al.; chitin binding fragments derived from chitinases isolated from plants such as Arabidopsis thaliana (Samac et al., Plant Physiol. 93: 907-914 (1990), tobacco (Lawton et al., Plant Mol. Biol. 19: 735-743 (1992)), fungi such as yeast (McCreath et al. Yeast 12: 501-504 (1996)), bacteria such as Bacillus circulans (Watanabe et al., J. Bacteriol. 1 74:408-414 (1992)), mammals, and insects. Chitin synthase which appears to be closely related to or substantially identical to human hyaluronic acid synthase (U.S. Pat. No. 6,492,150 to McDonald et al.), can be labeled and detected by tomography or NMR in vivo or in vitro. Polyclonal antibodies, monoclonal antibodies, Fab fragments, recombinant Fab polypeptides, Fv fragments, recombinant single-chain Fv polypeptides, and variations thereof which are specific for chitin can also be used as a probe. Anti-chitin antibodies have been disclosed in U.S. Pat. No. 5,004,699 to Winters. These probes can be labeled as above for tomographic or magnetic resonance imaging or with a relaxation agent such as a paramagnetic transition metal species which would enable magnetic resonance imaging by contrasting.

The labeled probes can be provided intravenously or injected directing into the area of the patient to be diagnosed.

Detail Description Paragraph - DETX (35):

[0075] In a third embodiment, the chitin is degraded with degradation enzymes such as chitinases. For example, U.S. Pat. Nos. 6,200,951, 6,399,571, and 6,372,212 to Gray et al., describes a human chitinase, the DNA encoding the chitinase, and fragments of the chitinase for detecting chitin, binding chitin, and treating fungal infections. These patents provide a detailed background regarding chitinase which enables the present invention.

Detail Description Paragraph - DETX (42):

[0082] U.S. Pat. No. 6,372,250 to Pardridge discloses an improved method for transporting the above therapeutic compounds across the BBB which uses liposomes which contain the therapeutic compound and which has disposed in the lipid membrane a plurality of agents which enable the liposomes to cross the BBB. These agents include insulin, transferrin, insulin-like growth factor, leptin, and low density lipoproteins. Alternatively, the agent is a peptidomimetic antibody which mimics the preceding peptides and which binds the receptor for the above proteins. The lipid membrane preferably further includes targeting agents which targets the liposome to the cells in the brain involved in synthesizing the chitin or the amyloid plaques or to the chitin or amyloid plaques per se. For example, the targeting agent can include the chitin binding sites of the chitinase identified in chitin binding lectins such as chitovibrin which is disclosed in U.S. Pat. Nos. 5,914,239 and 6,121,420, both to Laine; chitin binding fragments derived from human chitinase as disclosed in U.S. Pat. Nos. 6,399,571, 6,200,951 and 6,372,212, all to Gray et al., or U.S. Pat. Nos. 6,057,142 and 6,303,118, both to Aerts; chitin binding fragments derived from chitinases isolated from plants, fungi, bacteria, mammals, and insects. Alternatively, the targeting agent can include polyclonal antibodies, monoclonal antibodies, Fab fragments, recombinant Fab polypeptides, Fv fragments, recombinant single-chain Fv polypeptides, and variations thereof which are specific for chitin. In particular embodiments, the therapeutic compound can further include inhibitors or degraders of amyloid plaques.

Detail Description Paragraph - DETX (50):

[0090] Since chitin does not naturally occur in mammals and humans in the absence of a fungal or bacterial infection, animals and humans can be vaccinated with a chitin or chitin conjugate to stimulate an immune response to the chitin or conjugate. The immune response would include production of antibodies which would bind to chitin or conjugate wherever it might occur in the animal or human. The immune response might also include a cell-mediated response which would include macrophages which are capable of digesting the chitin. Human macrophages are known to contain a chitotriosidase (chitinase) activity. The immune response would protect the animal or human against diseases characterized by congo red-staining amyloid plaques because as the chitin is being formed, the vaccinated animal or human would be producing antibodies and macrophages in response. The antibodies and macrophages would lead to the degradation and removal of the chitin before it can lead to the formation of amyloid plaques.

Claims Text - CLTX (32):

31. The method of claim 30 wherein the chitinase is human chitinase and the mammal is human.

PGPUB-DOCUMENT-NUMBER: 20040175798

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040175798 A1

TITLE: Expression system for recombinant proteins

PUBLICATION-DATE: September 9, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Hoppe, Henry IV	Acton	MA	US	
Goodrick, Jason C.	San Francisco	CA	US	
Schilling, Bernhard M.	Syracuse	NY	US	

APPL-NO: 10/ 800602

DATE FILED: March 15, 2004

RELATED-US-APPL-DATA:

child 10800602 A1 20040315

parent continuation-of 10045507 20011107 US ABANDONED

non-provisional-of-provisional 60248806 20001115 US

US-CL-CURRENT: 435/69.1, 435/254.23

ABSTRACT:

A continuous fermentation process has been developed in Pichia pastoris (P. pastoris) in order to produce large quantities of recombinant human proteins. High expression levels have been demonstrated in continuous production of the enzyme by P. pastoris with a constitutive promoter in a 1.5-liter working volume fermenter using either glucose or glycerol as the carbon source. The fermentation could be extended for long periods of time with a excellent steady-state protein concentration and cell densities achieved. No proteolytic degradation of the enzyme was seen in the continuous fermentation mode.

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Summary of Invention Paragraph - BSTX (18):

[0016] The present invention provides methods for continuous high cell density fermentation system for the production of recombinant human proteins, including Chitinase, glucocerebrosidase, sphingomyelinase and others, preferably using constitutive promoters, such as the GAPDH promoter, in which proteolytic degradation of the product was reduced or even undetectable. Among other advantages, the proteins that are produced using the present system result in a high mannose carbohydrate moiety. While often a disadvantage, this glycosylation pattern is useful for the targeting of certain proteins to macrophages. In preferred embodiments of the invention, a continuous fermentation process is employed to produce recombinant human glucocerebrosidase with high mannose content.

Detail Description Paragraph - DETX (14):
Cloning and Selection of the Human Chitinase (hChitinase) Gene in P. pastoris

Detail Description Paragraph - DETX (51):
[0078] The feasibility of large-scale production of recombinant human chitinase using a constitutive Pichia pastoris expression system was demonstrated in a 21 L continuous stirred tank reactor (CSTR). A steady-state recombinant protein concentration in the supernatant of 250 mg/l was sustained for one month at a dilution rate of D=0.04 h.sup.-1 (equivalent to one volume exchange per day), enabling a volumetric productivity of 144 mg/l d (240 U/l d). The steady-state dry cell weight concentration in this high cell density culture reached 110 g/l. Considering safety and economical aspects, all large-scale cultivations were conducted without molecular oxygen supplementation. Conventional air sparging was used instead. The oxygen demand of the process was determined by off-gas analysis (OUR=4.8 g O.sub.2 1.sup.-1 h.sup.-1 with k.sub.La=846 h.sup.-1) and evaluated with regard to further reactor scale-up.

PGPUB-DOCUMENT-NUMBER: 20040121442

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040121442 A1

TITLE: Fungal chitinase, polynucleotide sequences encoding same, promoters of same, and uses thereof

PUBLICATION-DATE: June 24, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Chet, Ilan	Nes Ziona	IL		
Viterbo, Ada	Rehovot	IL		

APPL-NO: 10/ 475853

DATE FILED: November 3, 2003

PCT-DATA:
APPL-NO: PCT/IL02/00351
DATE-FILED: May 5, 2002
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/200, 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

A method of preventing or treating a disease or a condition associated with a chitin-containing organism in an individual, the method comprising administering to the individual a therapeutically effective amount of a pharmaceutical composition including as an active ingredient a polypeptide displaying an endochitinase activity.

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Summary of Invention Paragraph - BSTX (2):
[0001] The present invention is of recombinant fungal chitinases, polynucleotides encoding such chitinases, and uses thereof in treatment of human diseases caused by chitin-containing organisms, such as the fungus Candida albicans, and treatment of plant diseases caused by or associated with chitin-containing pathogens, such as fungal pathogens. The present invention is further of polynucleotides encoding regulatory sequences of genes encoding fungal chitinases and uses thereof in reducing susceptibility of plants to damage from stress conditions.

Summary of Invention Paragraph - BSTX (22):
[0021] In humans, studies suggest chitinases may play essential roles in immunity against chitin-containing pathogens, such as fungi, helminths, protozoans, etc. For example, chitinase activity has been demonstrated in human leukocytes (Escott et al., 1995. Infect. Immun. 63:4770), a chitinase (4-methylumbelliferyl-tetra-N-acetylchitotetraoside hydrolase) has been

isolated from human serum and rat liver (Overdijk et al., 1994. Glycobiology 4:797), a human chitotriosidase has been isolated from human spleen (Renkema et al., 1995. J. Biol. Chem. 270:2198), and human macrophage cDNA encoding a chitinase has been cloned (Boot et al., 1995. J. Biol. Chem. 270:26252).

US-PAT-NO: 6815183

DOCUMENT-IDENTIFIER: US 6815183 B1

TITLE: Plasmodium sp. chitinase

DATE-ISSUED: November 9, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Vinetz; Joseph M.	Galveston	TX	N/A	N/A

APPL-NO: 09/ 579383

DATE FILED: May 26, 2000

PARENT-CASE:

This application claims priority of U.S. Provisional Patent Application No. 60/136,508, filed May 28, 1999, and of U.S. Provisional Patent Application No. 60/180,051, filed Feb. 3, 2000.

US-CL-CURRENT: 435/69.3, 424/191.1 , 424/268.1 , 424/272.1 , 435/243 , 435/320.1 , 435/69.7 , 435/70.1 , 435/71.1 , 536/23.1 , 536/23.5 , 536/23.7

ABSTRACT:

The present invention is directed to isolated nucleic acid molecules encoding Plasmodium sp. chitinases. Expression vectors and host cells comprising the nucleic acid molecules are also provided, as well as methods for increasing or decreasing the expression of the chitinase in host cells. The invention further provides methods of screening a substance for the ability of the substance to modify chitinase function, and a method for isolating other chitinase molecules. DNA oligomers capable of hybridizing to the nucleic acid molecule encoding the chitinase are provided, which can be used to detect chitinase in a sample. An isolated Plasmodium sp. chitinase is also provided. Antibodies specific for the chitinase, and fragments thereof, are provided, as are compositions comprising the chitinase and a compatible carrier. The subject invention further provides methods of preventing infection of mosquitoes by Plasmodium sp. and methods of preventing transmission of malaria.

9 Claims, 42 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 17

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Brief Summary Text - BSTX (6):

A Plasmodium ookinete-secreted enzyme, chitinase (E.C. 3.2.1.14), has been demonstrated to be another target of blocking malaria transmission from humans to mosquitoes (Shahabuddin et al. 1993). Chitinases are found in prokaryotes and eukaryotes (Flach et al. 1992); their biologic roles include cell wall

modification (e.g. fungi (Kuranda and Robbins 1991), Entamoebae (Willagomez-Castro et al. 1992) and filaria parasites (Fuhrman and Piessens 1985)), carbon source degradation (e.g. Streptomyces spp. (Ni and Westpheling 1997; Robbins et al. 1988), Serratia marcescens (Roberts and Cabib 1982), and Vibrio spp. (Keyhani and Roseman 1996)), and plant and fungal host defense against chitin-containing pathogens (Flach et al. 1992). One other protozoan pathogen of man, Leishmania donovani, the agent of human visceral leishmaniasis, is known to use a chitinase in its life cycle (Schlein et al. 1991; Shakarian and Dwyer 1998). The Leishmania chitinase is thought to disrupt the sand fly cardiac valve, allowing amastigotes to be regurgitated from the midgut into the skin of the vertebrate host. The Leishmania chitinase is not thought to function in invasion of the arthropod vector per-se (Schlein et al. 1992). In contrast, Plasmodium chitinase is thought to be required for the parasite to invade the mosquito midgut after being taken up in a blood meal (Shahabuddin et al. 1993). Because of its critical biological function in the life cycle of the malaria parasite, the Plasmodium chitinase is a potential target for blocking transmission from the vertebrate host to the mosquito vector (Shahabuddin et al. 1993).

Drawing Description Text - DRTX (18):

FIG. 16 shows the homology model depicting the overlapping catalytic sites of PfCHT1, PgCHT1, and human chitotriosidase complexed with allosamidin. The three active sites are almost perfectly superimposable, with the exception of a novel pocket found in PgCHT1, seen at lower right. The models were built by using the structure of hevamine complexed with allosamidin as a template.

Detailed Description Text - DETX (198):

Molecular Modeling of PfCHT1. PfCHT1 is predicted to have an (.alpha..beta.).sub.8 triose isomerase barrel structure typical of family 18 chitinases (Terwisscha van Scheltinga et al. 1996). A majority of the active-site residues of PfCHT1 are common to either hevamine or Serratia marcescens chitinase ChiA, for which crystal structures are available (Terwisscha van Scheltinga et al. 1996; Perrakis et al. 1994). The Plasmodium chitinases are unique in that they have a Gly for Phe/Met (hevamine/ChiA, respectively) change at a position (353 for PfCHT1, 405 for PgCHT1) that is highly conserved among other family 18 chitinases. This position is in a critical area at the base of the catalytic site (FIG. 16) and may impart a unique structure. To explore further the potential implication of this position as a site for selective drug targeting, homology models were built for PfCHT1, PgCHT1, and human chitotriosidase (Boot et al. 1995) (FIG. 16). Although the Gly for Phe/Met change substantially enlarges the base of the catalytic pocket in PgCHT1, a complementary Tyr.sup.309 in PfCHT1 on the .beta.-7 strand compensates for the missing volume, resulting in an almost perfect overlap of the catalytic pocket with that of human chitotriosidase. In contrast, the I.sup.361 change in the PgCHT1 .beta.7 strand does not fully compensate for the Gly for Phe/Met change. The resulting unique pocket distinguishes PgCHT1 from PfCHT1 and may explain the differential sensitivity of PfCHT1 and PgCHT1 to allosamidin. In the model, allosamidin does not appear to contact Gly.sup.405 of PgCHT1 but does appear to contact Tyr.sup.309 in PfCHT1.

US-PAT-NO: 6794150

DOCUMENT-IDENTIFIER: US 6794150 B2

TITLE: Assay for YKL-40 as a marker for degradation of mammalian connective tissue matrices

DATE-ISSUED: September 21, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Price; Paul A.	La Jolla	CA	N/A	N/A
Johansen; Julia S.	Copenhagen	N/A	N/A	DK

APPL-NO: 09/ 215077

DATE FILED: December 18, 1998

PARENT-CASE:

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. Ser. No. 08/581,527, filed on Apr. 17, 1996, now U.S. Pat. No. 5,935,798 which is a 371 of PCT/US94/07754, filed on Jul. 8, 1994, which is a continuation-in-part of U.S. Ser. No. 08/089,989, filed on Jul. 9, 1993, now abandoned, which are all incorporated herein by reference in their entirety for all purposes.

US-CL-CURRENT: 435/7.23, 435/4 , 435/7.1 , 435/7.4 , 435/7.92 , 435/975 , 436/501 , 436/518 , 436/525 , 436/63 , 436/64 , 436/808 , 436/811 , 436/813 , 530/387.9 , 530/388.1 , 530/388.85 , 530/389.7

ABSTRACT:

The invention is a method of identifying the presence of, and monitoring, a disease state in a mammal which is associated with degradation of connective tissue in the mammal. The method detects and determines whether diagnostically or prognostically significant levels of YKL-40 protein and/or YKL-40 peptide are present in a biological sample. The method can be used, for example, to identify the presence of inflammatory joint disease or degeneration of connective tissue in organs. Serum YKL-40 levels as detected and quantified by the invention method are also suggestive of the prognosis for the length of survival in breast cancer patients following recurrence and/or metastasis of their cancers.

7 Claims, 12 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 10

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Other Reference Publication - OREF (26):
EMHUM2 Database Accession No. HSU58514 (Jul. 26, 1996) from Grossman et al.,

"Cloning of a Novel Lymphoid Restricted Human Chitinase and Localization to 1p13.3 (unpublished)"--abstract.

Other Reference Publication - OREF (27):
EMHUM2 Database Accession No. HSU58515 (Jul. 26, 1996) from Grossman et al., "Cloning of a Novel Lymphoid Restricted Human Chitinase and Localization ot 1p13.3 (unpublished)"--abstract.

US-PAT-NO: 6789069

DOCUMENT-IDENTIFIER: US 6789069 B1

TITLE: Method for enhancing knowledge discovered from biological data using a learning machine

DATE-ISSUED: September 7, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnhill; Stephen	Savannah	GA	N/A	N/A
Guyon; Isabelle	Berkeley	CA	N/A	N/A
Weston; Jason	New York	NY	N/A	N/A

APPL-NO: 09/ 633850

DATE FILED: August 7, 2000

PARENT-CASE:

RELATED APPLICATIONS

This appln claims benefit of 60/161,806 Oct. 27, 1999 and claims benefit of 60/168,703 Dec. 27, 1999 and claims benefit of 60/184,596 Feb. 24, 2000 and claims benefit of 60/191,219 Mar. 22, 2000 U.S. Pat. No. 6,658,395 and is a CIP of Ser. No. 09/578,011 May 24, 2000 now U.S. Pat. No. 6,658,395 which claims benefit of 60/135,715 May 25, 1999 and is a CIP of Ser. No. 09/568,301 May 9, 2000 U.S. Pat. No. 6,427,141 which is a CON of Ser. No. 09/303,387 May 1, 1999 U.S. Pat. No. 6,128,608 and is a CON of Ser. No. 09/305,345 May 1, 1999 U.S. Pat. No. 6,157,921 and is a CON of Ser. No. 09/303,386 May 1, 1999 abandoned and is a CON of Ser. No. 09/303,389 May 1, 1999 abandoned which claims benefit of 60/083,961 May 1, 1998

US-CL-CURRENT: 706/12, 706/45

ABSTRACT:

A learning machine is used to extract useful information from vast quantities of biological data. The method includes pre-processing of training data and test data to add dimensionality or to identify missing or erroneous data points. The training data is used to train the learning machine after which the success of the training is tested using the test data. The test output is pre-processed to determine whether the knowledge discovered from the pre-processed test data set is desirable. After the training has been confirmed, live biological data can be pre-processed then input into the trained learning machine for extraction of useful information. In the preferred embodiment, the learning machine is one or more support vector machines.

51 Claims, 54 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 33

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Detailed Description Text - DETX (198):
In the case of human chitotriosidase, one needs to proceed by analogy with another homologous protein of the same family whose role in another cancer is under study: another chitinase (BRP39) was found to play a role in breast cancer. Cancer cells overproduce this chitinase to survive apoptosis (Aronson, 1999). Important increased chitotriosidase activity has been noticed in clinical studies of Gauchers disease patients, an apparently unrelated condition. To diagnose that other disease the chitotriosidase enzyme can be very sensitively measured. The plasma or serum prepared from less than a droplet of blood is highly sufficient for the chitotriosidase measurement (Aerts, 1996). This opens the door to a possible new diagnosis test for colon cancer as well.

Detailed Description Paragraph Table - DETL (4):
TABLE 2 QT_clust clusters selected with RFE. The higher the cluster rank (Rk), the more important the cluster. Min correl is the minimum correlation coefficient between cluster elements. GAN = Gene Accession Number. Rk Min correl GAN Description 1 0.82 X54163 TROPONIN I, CARDIAC MUSCLE (HUMAN);contains element MER22 repetitive element D23672 Human mRNA for biotin-[propionyl-CoA- carboxylase(ATP-hydrolysing)] ligase, complete cds. Y00970 2 0.82 T51023 75127 HEAT SHOCK PROTEIN HSP 90- BETA (HUMAN). T69446 82983 EUKARYOTIC INITIATION FACTOR 4A-I (HUMAN);. R37428 26100 Human unknown protein mRNA, partial cds. H89087 253224 SPLICING FACTOR SC35 (Homo sapiens) R96357 197929 POLYADENYLATE-BINDING PROTEIN (Xenopus laevis) T96873 121343 HYPOTHETICAL PROTEIN IN TRPE 3REGION (Spirochaeta aurantia) H72234 213492 DNA-(APURINIC OR APYRIMIDINIC SITE) LYASE (HUMAN);. 3 0.83 T85247 111192 CYTOCHROME C OXIDASE POLYPEPTIDE VIC PRECURSOR (HUMAN);. R08021 127104 INORGANIC PYROPHOSPHATASE (Bos taurus) M22760 Homo sapiens nuclear-encoded mitochondrial cytochrome c oxidase Va subunit mRNA, complete cds. 4 0.84 T94579 119384 Human chitotriosidase precursor mRNA, complete cds. T83361 116665 GAMMA INTERFERON INDUCED MONOKINE PRECURSOR (Homo sapiens) R89377 196061 NEDD5 PROTEIN (Mus musculus) 5 0.85 R51749 39237 TRANS-ACTING TRANSCRIPTIONAL PROTEIN ICP4 (Equine herpesvirus type 1) R10620 128901 TYROSINE-PROTEIN KINASE CSK (Homo sapiens) H29483 49967 INTERCELLULAR ADHESION MOLECULE-2 PRECURSOR (HUMAN);. 6 0.82 X55187 Human mRNA for alpha-actinin, partial cds. X74295 H.sapiens mRNA for alpha 7B integrin. R48303 153505 TYROSINE RICH ACIDIC MATRIX PROTEIN (Bos taurus) X86693 H.sapiens mRNA for hevin like protein. H06524 44386 GELSOLIN PRECURSOR, PLASMA (HUMAN);. 7 0.87 H61410 211590 PLATELET GLYCOPROTEIN IV (Homo sapiens) H67764 229939 ESTROGEN SULFOTRANSFERASE (Bos taurus) U06698 Human neuronal kinesin heavy chain mRNA, complete cds. R39209 23464 HUMAN IMMUNODEFICIENCY VIRUS TYPE I ENHANCER-BINDING PROTEIN 2 (Homo sapiens) R39209 23464 HUMAN IMMUNODEFICIENCY VIRUS TYPE I ENHANCER-BINDING PROTEIN 2 (Homo sapiens) 8 0.82 R10066 128808 PROHIBITIN (Homo sapiens) U09564 Human serine kinase mRNA, complete cds. R62549 138906 PUTATIVE SERINE/THREONINE- PROTEIN KINASE B0464.5 IN CHROMOSOME III (Caenorhabditis elegans)

Detailed Description Paragraph Table - DETL (6):
TABLE 4 The 7 top ranked genes discovered by the methods of the present invention, in order of increasing importance. Rk: rank. Sgn: sign of correlation with the target separation, - for over-expressed in most cancer tissues; + for over-expressed in most normal tissues; GAN: Gene Accession Number; The possible function is derived from a keyword search involving "colon cancer" or "cancer" and some words in the gene description. Possible function/relation to Rk Sgn GAN Description colon cancer 1 - H08393 COLLAGEN Collagen is involved in cell ALPHA 2(XI) adhesion. Colon carcinoma cells CHAIN (Homo have collagen degrading activity sapiens) as part of the

metastatic process. 2 - M59040 Human cell CD44 is upregulated when colon adhesion adenocarcinoma tumor cells molecule (CD44) transit to the metastatic state. mRNA, complete cds. 3 - T94579 Human Another chitinase (BRP39) was chitotriosidase found to play a role in breast precursor mRNA, cancer. Cancer cells overproduce complete cds. this chitinase to survive apoptosis. 4 + H81558 PROCYCLIC It was shown that patients FORM infected by Trypanosoma (a SPECIFIC colon parasite) develop POLYPEPTIDE resistance against colon cancer. B1-ALPHA PRECURSOR (Trypanosoma brucei brucei) 5 + R88740 ATP ATP synthase is an enzyme that SYNTHASE helps build blood vessels that COUPLING feed the tumors. FACTOR 6, MITO- CHONDRIA L PRECURSOR (HUMAN) 6 - T62947 60S May play a role in controlling RIBOSOMAL cell growth and proliferation PROTEIN L24 through the selective translation (Arabidopsis of particular classes of mRNA. thaliana) 7 + H64807 PLACENTAL Diminished status of folate has FOLATE been associated with enhanced TRANSPORTER risk of colon cancer. (Homo sapiens)

Detailed Description Paragraph Table - DETL (8):

TABLE 6 SVM top ranked clusters when using all 62 tissues. Clusters are built around the best genes with threshold .theta. = 0.75.The higher the cluster rank (Rk), the more "relevant" the cluster should be. Min correl is the minimum correlation coefficient between cluster elements. Sgn: sign of correlation with the target separation, - for over-expressed in most cancer tissues; + for over-expressed in most normal tissues; GAN: Gene Accession Number. The cluster centers are preceded by a star. None of the genes seem to be tissue composition related. Min Rk correl Sgn GAN Description 1 0.75 - *H08393 COLLAGEN ALPHA 2(XI) CHAIN (Homo sapiens) - T48804 40S RIBOSOMAL PROTEIN S24 (HUMAN) - T51529 ELONGATION FACTOR 1-DELTA (Artemia salina) 2 0.61 - *M59040 Human cell adhesion molecule (CD44) mRNA, complete cds. - H04802 DIHYDROPRYRIDINE-SENSITIVE L- TYPE, SKELETAL MUSCLE CALCIUM CHANNEL GAMMA SUBUNIT (Homo sapiens) - T65740 SINGLE-STRANDED DNA BINDING PROTEIN P9 PRECURSOR (Mus musculus) - L39874 Homo sapiens deoxycytidylate deaminase gene, complete cds. - R44740 DUAL SPECIFICITY MITOGEN- ACTIVATED PROTEIN KINASE KINASE 1 (Xenopus laevis) 3 0.54 - *T94579 Human chitotriosidase precursor mRNA, complete cds. - T63539 INHIBIN BETA A CHAIN PRECURSOR (Mus musculus) - T54360 GRANULINS PRECURSOR (HUMAN). + X17273 Human HLA G (HLA 6.0) mRNA for non classical class I transplantation antigen. + T57882 MYOSIN HEAVY CHAIN, NONMUSCLE TYPE A (Homo sapiens) - R89377 NEDD5 PROTEIN (Mus musculus) - M19283 Human cytoskeletal gamma-actin gene, complete cds. - T83361 GAMMA INTERFERON INDUCED MONOKINE PRECURSOR (Homo sapiens) - H66786 ESTROGEN SULFOTRANSFERASE (Bos taurus) - T51849 TYROSINE-PROTEIN KINASE RECEPTOR ELK PRECURSOR (Rattus norvegicus) - T86444 PROBABLE NUCLEAR ANTIGEN (Pseudorabies virus) 4 1 + *H81558 PROCYCLIC FORM SPECIFIC POLYPEPTIDE B1-ALPHA PRECURSOR (Trypanosoma brucei brucei) 5 0.81 + *R88740 ATP SYNTHASE COUPLING FACTOR 6, MITOCHONDRIAL PRECURSOR (HUMAN);. + T54670 P13621 ATP SYNTHASE OLIGOMYCIN SENSITIVITY CONFERRAL PROTEIN PRECURSOR, MITOCHONDRIAL. 6 0.61 - *T62947 60S RIBOSOMAL PROTEIN L24 (Arabidopsis thaliana) - T61609 LAMININ RECEPTOR (HUMAN);. - T70062 Human nuclear factor NF45 mRNA, complete cds. - U14971 Human ribosomal protein S9 mRNA, complete cds. - T57619 40S RIBOSOMAL PROTEIN S6 (Nicotiana tabacum) - U30825 Human splicing factor SRp30c mRNA, complete cds. - L10284 Homo sapiens integral membrane protein, calnexin, (IP90) mRNA, complete cds. - D00763 PROTEASOME COMPONENT C9 (HUMAN);. - T58861 60S RIBOSOMAL PROTEIN L30E (Kluyveromyces lactis) 7 1 + *H64807 PLACENTAL FOLATE TRANSPORTER (Homo sapiens)

US-PAT-NO: 6760715

DOCUMENT-IDENTIFIER: US 6760715 B1

TITLE: Enhancing biological knowledge discovery using multiples support vector machines

DATE-ISSUED: July 6, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Barnhill; Stephen	Savannah	GA	N/A	N/A
Guyon; Isabelle	Berkeley	CA	N/A	N/A
Weston; Jason	New York	NY	N/A	N/A

APPL-NO: 09/ 633616

DATE FILED: August 7, 2000

PARENT-CASE:

RELATED APPLICATIONS

The application is a continuation-in-part of U.S. patent application Ser. No. 09/303,386; filed May 1, 1999 now abandoned; Ser. No. 09/303,387, now U.S. Pat. No. 6,128,608; 09/303,389 now abandoned, Ser. No. 09/305,345, now U.S. Pat. No. 6,157,921; all filed May 1, 1999; and U.S. patent application Ser. No. 09/568,301, now U.S. Pat. No. 6,427,141, filed May 9, 2000; and U.S. patent application Ser. No. 09/578,011, now U.S. Pat. No. 6,658,395, filed May 24, 2000, and also claims the benefit of U.S. Provisional Patent Application No. 60/161,806, filed Oct. 27, 1999; of U.S. Provisional Patent Application No. 60/168,703, filed Dec. 2, 1999; of U.S. Provisional Patent Application No. 60/184,596, filed Feb. 24, 2000; and of U.S. Provisional Patent Application Ser. No. 60/191,219, filed Mar. 22, 2000.

US-CL-CURRENT: 706/16, 706/12 , 706/20 , 706/25 , 706/45

ABSTRACT:

Multiple support vector machines are used to extract useful information from vast quantities of biological data. The method includes pre-processing of training data and test data to add dimensionality or to identify missing or erroneous data points. The training data is used to train the learning machine after which the success of the training is tested using the test data. The test output is pre-processed to determine whether the knowledge discovered from the pre-processed test data set is desirable and to identify which of the multiple support vector machines provides the optimal solution. After the training has been confirmed, live biological data can be pre-processed then input into the identified support vector machine that provides the optimal solution for extraction of useful information.

22 Claims, 54 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 33

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Detailed Description Text - DETX (198):
In the case of human chitotriosidase, one needs to proceed by analogy with another homologous protein of the same family whose role in another cancer is under study: another chitinase (BRP39) was found to play a role in breast cancer. Cancer cells overproduce this chitinase to survive apoptosis (Aronson, 1999). Important increased chitotriosidase activity has been noticed in clinical studies of Gauchers disease patients, an apparently unrelated condition. To diagnose that other disease the chitotriosidase enzyme can be very sensitively measured. The plasma or serum prepared from less than a droplet of blood is highly sufficient for the chitotriosidase measurement (Aerts, 1996). This opens the door to a possible new diagnosis test for colon cancer as well.

Detailed Description Paragraph Table - DETL (4):
TABLE 2 Rk Min correl GAN Description 1 0.82 X54163 TROPONIN I, CARDIAC MUSCLE (HUMAN); contains element MER22 repetitive element D23672 Human mRNA for biotin-[propionyl-CoA- carboxylase(ATP-hydrolysing)] ligase, complete cds. Y00970 2 0.82 T51023 75127 HEAT SHOCK PROTEIN HSP 90- BETA (HUMAN). T69446 82983 EUKARYOTIC INITIATION FACTOR 4A-I (HUMAN);. R37428 26100 Human unknown protein mRNA, partial cds. H89087 253224 SPLICING FACTOR SC35 (Homo sapiens) R96357 197929 POLYADENYLATE-BINDING PROTEIN (Xenopus laevis) T96873 121343 HYPOTHETICAL PROTEIN IN TRPE 3REGION (Spirochaeta aurantia) H72234 213492 DNA-(APURINIC OR APYRIMIDINIC SITE) LYASE (HUMAN);. 3 0.83 T85247 111192 CYTOCHROME C OXIDASE POLYPEPTIDE VIC PRECURSOR (HUMAN);. R08021 127104 INORGANIC PYROPHOSPHATASE (Bos taurus) M22760 Homo sapiens nuclear-encoded mitochondrial cytochrome c oxidase Va subunit mRNA, complete cds. 4 0.84 T94579 119384 Human chitotriosidase precursor mRNA, complete cds. T83361 116665 GAMMA INTERFERON INDUCED MONOKINE PRECURSOR (Homo sapiens) R89377 196061 NEDD5 PROTEIN (Mus musculus) 5 0.85 R51749 39237 TRANS-ACTING TRANSCRIPTIONAL PROTEIN ICP4 (Equine herpesvirus type 1) R10620 128901 TYROSINE-PROTEIN KINASE CSK (Homo sapiens) H29483 49967 INTERCELLULAR ADHESION MOLECULE-2 PRECURSOR (HUMAN);. 6 0.82 X55187 Human mRNA for alpha-actinin, partial cds. X74295 H. sapiens mRNA for alpha 7B integrin. R48303 153505 TYROSINE RICH ACIDIC MATRIX PROTEIN (Bos taurus) X86693 H. sapiens mRNA for hevin like protein. H06524 44386 GELSOLIN PRECURSOR, PLASMA (HUMAN);. 7 0.87 H61410 211590 PLATELET GLYCOPROTEIN IV (Homo sapiens) H67764 229939 ESTROGEN SULFOTRANSFERASE (Bos taurus) U06698 Human neuronal kinesin heavy chain mRNA, complete cds. R39209 23464 HUMAN IMMUNODEFICIENCY VIRUS TYPE I ENHANCER-BINDING PROTEIN 2 (Homo sapiens) R39209 23464 HUMAN IMMUNODEFICIENCY VIRUS TYPE I ENHANCER-BINDING PROTEIN 2 (Homo sapiens) 8 0.82 R10066 128808 PROHIBITIN (Homo sapiens) U09564 Human serine kinase mRNA, complete cds. R62549 138906 PUTATIVE SERINE/THREONINE- PROTEIN KINASE B0464.5 IN CHROMOSOME III (Caenorhabditis elegans) QT_clust clusters selected with RFE. The higher the cluster rank (Rk), the more important the cluster. Min correl is the minimum correlation coefficient between cluster elements. GAN=Gene Accession Number.

Detailed Description Paragraph Table - DETL (6):
TABLE 4 Possible function/relation to Rk Sgn GAN Description colon cancer 1 - H08393 COLLAGEN Collagen is involved in cell ALPHA 2(XI) adhesion. Colon carcinoma CHAIN (Homo cells have collagen degrading sapiens) activity as part of the metastatic process. 2 - M59040 Human cell CD44 is upregulated when adhesion molecule colon adenocarcinoma tumor (CD44) mRNA, cells transit to the complete cds. metastatic state. 3 - T94579 Human Another chitinase (BRP39) chitotriosidase was found to play a role in precursor mRNA, breast cancer. Cancer cells complete cds. overproduce this chitinase to survive apoptosis.

4 + H81558 PROCYCLIC It was shown that patients FORM SPECIFIC infected by Trypanosoma (a POLYPEPTIDE B1- colon parasite) develop ALPHA resistance against colon PRECURSOR cancer. (Trypanosoma brucei brucei) 5 + R88740 ATP SYNTHASE ATP synthase is an enzyme COUPLING that helps build blood vessels FACTOR 6, that feed the tumors. MITO- CHONDRIAL PRECURSOR (HUMAN) 6 - T62947 60S RIBOSOMAL May play a role in controlling PROTEIN L24 cell growth and proliferation (Arabidopsis through the selective thaliana) translation of particular classes of mRNA. 7 + H64807 PLACENTAL Diminished status of folate FOLATE has been associated with TRANSPORTER enhanced risk of colon cancer. (Homo sapiens) The 7 top ranked genes discovered by the methods of the present invention, in order of increasing importance. Rk: rank. Sgn: sign of correlation with the target separation, - for over-expressed in most cancer tissues; + for over-expressed in most normal tissues; GAN: Gene Accession Number; The possible function is derived from a keyword search involving "colon cancer" or "cancer" and some words in the gene description.

Detailed Description Paragraph Table - DETL (8):

TABLE 6 Min Rk correl Sgn GAN Description 1 0.75 - * H08393 COLLAGEN ALPHA 2(XI) CHAIN (Homo sapiens) - T48804 40S RIBOSOMAL PROTEIN S24 (HUMAN). - T51529 ELONGATION FACTOR 1-DELTA (Artemia salina) 2 0.61 - * M59040 Human cell adhesion molecule (CD44) mRNA, complete cds. - H04802 DIHYDROPRYRIDINE-SENSITIVE L- TYPE, SKELETAL MUSCLE CALCIUM CHANNEL GAMMA SUBUNIT (Homo sapiens) - T65740 SINGLE-STRANDED DNA BINDING PROTEIN P9 PRECURSOR (Mus musculus) - L39874 Homo sapiens deoxycytidylate deaminase gene, complete cds. - R44740 DUAL SPECIFICITY MITOGEN- ACTIVATED PROTEIN KINASE KINASE 1 (Xenopus laevis) 3 0.54 - * T94579 Human chitotriosidase precursor mRNA, complete cds. - T63539 INHIBIN BETA A CHAIN PRECURSOR (Mus musculus) - T54360 GRANULINS PRECURSOR (HUMAN). + X17273 Human HLA G (HLA 6.0) mRNA for non classical class I transplantation antigen. + T57882 MYOSIN HEAVY CHAIN, NON- MUSCLE TYPE A (Homo sapiens) - R89377 NEDD5 PROTEIN (Mus musculus) - M19283 Human cytoskeletal gamma-actin gene, complete cds. - T83361 GAMMA INTERFERON INDUCED MONOKINE PRECURSOR (Homo sapiens) - H66786 ESTROGEN SULFOTRANSFERASE (Bos taurus) - T51849 TYROSINE-PROTEIN KINASE RECEPTOR ELK PRECURSOR (Rattus norvegicus) - T86444 PROBABLE NUCLEAR ANTIGEN (Pseudorabies virus) 4 1 + * H81558 PROCYCLIC FORM SPECIFIC POLYPEPTIDE B1-ALPHA PRE- CURSOR (Trypanosoma brucei brucei) 5 0.81 + * R88740 ATP SYNTHASE COUPLING FACTOR 6, MITOCHONDRIAL PRECURSOR (HUMAN);. + T54670 P13621 ATP SYNTHASE OLIGOMYCIN SENSITIVITY CONFERRAL PROTEIN PRE- CURSOR, MITOCHONDRIAL. 6 0.61 - * T62947 60S RIBOSOMAL PROTEIN L24 (Arabidopsis thaliana) - T61609 LAMININ RECEPTOR (HUMAN);. - T70062 Human nuclear factor NF45 mRNA, complete cds. - U14971 Human ribosomal protein S9 mRNA, complete cds. - T57619 40S RIBOSOMAL PROTEIN S6 (Nicotiana tabacum) - U30825 Human splicing factor SRp30c mRNA, complete cds. - L10284 Homo sapiens integral membrane protein, calnexin, (IP90) mRNA, complete cds. - D00763 PROTEASOME COMPONENT C9 (HUMAN);. - T58861 60S RIBOSOMAL PROTEIN L30E (Kluyveromyces lactis) 7 1 + * H64807 PLACENTAL FOLATE TRANSPORTER (Homo sapiens) SVM top ranked clusters when using all 62 tissues. Clusters are built around the best genes with threshold .theta. = 0.75. The higher the cluster rank (Rk), the more "relevant" the cluster should be. Min correl is the minimum correlation coefficient between cluster elements. Sgn: sign of correlation with the target separation, - for over-expressed in most cancer tissues; + for over-expressed in most normal tissues; GAN: Gene Accession Number. The cluster centers are preceded by a star. None of the genes seem to be tissue composition related.

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2931	chitinase$1 or chitotriosidase$1	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:04
L2	47	1 near4 human	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:11
L3	75	1 same (culture adj medi$4)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:20
L4	101	1 same (cosemetic$1 or dental or toothpaste$1 or food)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:21
L5	230	1 same antifung$	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:21
L6	19	5 same (human or mammal$)	US-PGPUB; USPAT	OR	OFF	2004/12/23 08:22

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TITLE: Transgenic plants for mitigating introgression of genetically engineered genetic traits

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INVENTOR-INFORMATION:

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child 10774388 A1 20040210

parent continuation-in-part-of 09889737 20010720 US ABANDONED

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parent a-371-of-international PCT/IL00/00046 20000124 WO PENDING

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
IL	128353	1999IL-128353	February 3, 1999

US-CL-CURRENT: 800/278

ABSTRACT:

Genetic mechanisms for mitigating the effects of introgression of a genetically engineered genetic trait of a cultivated crop to an undesirable, interbreeding related species.

[0001] This is a continuation-in-part of U.S. patent application Ser. No. 09/889,737, filed 24 Jan. 2000, which is a U.S. National Phase application of PCT/1L00/00046, filed 24 Jan., 2000, which claims priority from Israeli Patent Application No. 128353, filed 3 Feb., 1999.

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Detail Description Table CWU - DETL (4):
5TABLE 5 Examples of primary genes inserted into corn for potentially commercial purposes - USA field releases Type of gene/phenotype Gene or protein encoded APHIS.sup.#a Herbicide Resistance Glufosinate pat 03-301-04 Glyphosate EPSP synthase 03-301-13 Glyphosate oxireductase 96-099-02 Imidazolinone group CBI 03-301-08 Protox inhibitors CBI 03-021-02 Isoxazole

group CBI 02-070-19 Chloroacetanilide group CBI 00-073-06 Cyanamide CBI 00-021-03 Dalapon CBI 00-024-02 Insect Resistance Coleopteran CBI 03-301-13 Bt 03-140-03 Lepidopteran CBI 03-301-04 Cry 1A Bt 03-022-01 Disease Resistance (fungi) Rhizoctonia CBI 03-202-12 Fusarium ear rot CBI 03-021-02 Southern corn leaf blight CBI 02-079-13 CBI CBI 01-123-01 Botrytis CBI 01-131-01 Aspergillus Chitinase 00-096-04 Chitinase + glucanase 98-322-03 Smut CBI 00-021-03 Gray leaf spot CBI 99-028-01 Northern corn leaf blight CBI 99-032-02 Septoria CBI 99-032-02 Helminthosporium CBI 98-292-03 Alternaria CBI 98-078-15 Cercospora antifungal + ribosome 96-106-04 inactivating proteins Disease Resistance (Bacterial) CBI CBI 01-123-01 Disease Resistance (viral) CBI CBI 01-123-01 MDMV MDMVcoat protein 95-328-04 MCMV MCMV coat protein 94-048-03 Agronomic Quality.sup.a Seed Quality CBI 03-303-04 Environmental stress CBI 03-290-03 Yield increase CBI 03-288-10 Germination increase CBI 03-276-06 Drought tolerant CBI 03-276-10 Seed color altered BI regulatory gene 03-258-07 Male sterile B cell lymphoma related gene X 03-121-02 Male sterility protein 03-114-05 barnase 03-077-15 Fertility altered Aldehyde dehydrogenase 03-091-19 rf2a restorer genet TURF13 mitochondrial + 03-091-12 aldehyde dehydrogenase adenine methylase + CBI 03-015-01 Senescence altered CBI 03-052-56 Endosperm DNA cyclin dependent kinase + 03-050-08 synthesis altered retinoblastoma related protein Development altered Mu 1 transposable element 02-3354-01 Enhanced photosynthesis CBI 98-128-22 Cold tolerant CBI 02-262-05 Storage protein altered CBI 02-072-10 Increased Stalk strength 02-023-01 Tryptophan level CBI 02-070-26 Gene expression altered glutathione transferase 02-032-20 Carbohydrate metabolism CBI 98-041-05 altered Product Quality.sup.a Oil profile altered CBI 03-272-03 acetyl-CoA-carboxylase 98-093-05 Protein quality CBI 03-258-14 glutenin 02-087-01 homoserine dehydrogenase + 95-291-19 spertokinase zein storage protein 95-291-07 Lysine level altered CBI 03-258-15 aspartokinase and 99-305-01 dihydropicolinate synthase Animal feed quality CBI 03-022-02 improved Phytate reduced CBI 02-302-07 Starch metabolism starch synthase 02-289-07 altered ADP glucose pyrophosphorylase 02-261-03 isoamylase debranching enzyme 99-302-11 Levansucrase 98-238-04 amylase 98-139-01 Fumonisin degradation CBI 02-023-02 methionine increased CBI 99-160-03 storage protein 98-070-01 Novel protein CBI 99-055-11 (Pharmaceutical) Reduced lignin Omethyltransferase 98-215-05 Colored seed anthocyanin regulatory gene 97-189-05 Pharmaceutical traits Man & mouse CBI genes 03-143-01 CBI 03-086-01 Hepatitis B virus surface antigen 01-187-01 G-glycoprotein 98-117-04 .alpha. and .beta. human 98-117-01 subunit hemoglobins Enterotoxin B 01-190-01 human serum albumin 98-117-03 antibody 99-271-04 human procollagen 98-117-02 Industrial enzyme Turkey laccase 02-113-09 produced Novel protein CBI 02-081-10 Pentadiplandra brazzein 02-081-13 Cecropin 99-106-11 lipase 99-091-04 Polymer production Ketothiolase + acetoacetylCoA 01-150-01 reductase + polyhydrobutyrate synthase .sup.a-Some product quality traits listed as agronomic traits by APHIS